APPENDIX – IV

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MAHARSHI DAYANAND SARASWATI UNIVERSITY AJMER
FORMAT FOR OUTLINE OF PROPOSED RESEARCH WORK

1. Name of scholar (In English): **PRATIMA YADAV**

   (In Hindi): प्रतिमायादव

2. Title of the research work

   (In English): **PESTICIDE RESIDUES IN CATTLE FEED AND MILK – A STUDY OF AJMER, RAJASTHAN**

   (In Hindi): अजमेर राजस्थान के पशु आहार व दुग्ध में कीटनाशक अवशेष का अध्ययन

3. Location:

   A) Department of Environmental Science, Maharshi Dayanand Saraswati University, Ajmer

   Geographical Area of Investigation, Ajmer, Rajasthan

4. Introduction Importance of Proposed Investigation, Objectives.
5. Review (Work already done on the subject of proposed research study)
6. Research Gaps identified in the proposed field of investigation. (Based on the review)
7. Major Hypothesis, if any
8. Research Methodology.
9. List of References cited.

Signature of the Candidate with date Pratima yadav 15/09/2016

Outline Approved

15/9/16

Name and Signature of supervisor with date & seal

Prof. Subroto Dutta
Department of Environmental Science
M.D.S. University

MAHARSHI DAYANAND SARASWATI UNIVERSITY AJMER

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3. GEOGRAPHCAL AREA OF INVESTIGATION

The area under investigation, Ajmer is one of the developing cities known as ‘Heart of Rajasthan’. From ancient time it has been known as a political centre & British also made this as a political headquarter. It is also known as the main education centre. Besides this it has a combination of religious harmony due to famous mosque Dargah Sharif and Pushkar. The district is triangular in shape.

The area under investigation is centrally situated city of Rajasthan lies between 26°25’ to 26°29’ N latitudes and 74°37’ to 74°42’ E longitudes. Area of Ajmer is 8481.40 Sq. km and area of the Ajmer city is 241.56 Sq. km. It is represented by Aravalli hillocks, sand dunes, agricultural fields and fresh water bodies like Anasagar, Foysagar, Pushkar and Budha Pushkar.The entire length of Aravalli is about 692 km running from North-East (near Delhi) to South-West of Gujarat. The central Aravalli covers the entire Ajmer region. The region has a thickness of argillaceous rocks. The district is endowed with a large number of non-metallic minerals e.g. asbestos, beryl emerald, feldspar, garnet, mica, and vermiculite4.17% of total area is covered under foresest (Wikipedia).

4. INTRODUCTION

IMPORTANCE OF RESEARCH WORK

Pesticides used in agriculture to control pests such as insects, weeds and plant diseases have been subject to considerable legislative, regulatory and consumer scrutiny over the past few decades. Pesticides are toxic in nature; since many pesticides may potentially leave residues on foods available for human consumption, there is much concern regarding the potential health risks of pesticides in the human diet. The problem of contamination of our food commodities especially milk and milk products by pesticide residues constitutes one of the most serious challenges to public health. The information on the levels of pesticide residues occurring in food commodities is essential and can be obtained through regular monitor procedures. The analytical surveys not only provide the current data on pesticide residue Contamination but also serve to indicate whether or not the principles of good agricultural practices are being followed. Moreover, they also provide information regarding the Government polices in context of the restriction or ban on the use of certain persistent chemicals which may pose serious hazards to environment and human. The residues monitoring programmes are the key means of ensuring compliance with regulations. These also create a database to help in assessing the levels of pesticide residue and the levels of residue intake. Many countries including India in the world have established analytical laboratories to monitor residue levels in foodarticles (Sharma et al.(2007).

Pesticide pollution to the local environment also affects the lives of birds, wildlife, domestic animals, fish and livestock. Therefore, the determination of pesticide residues in the environment and in foods is necessary for ensuring that human exposure to contaminants especially by dietary intake does not exceed acceptable levels for health.

PESTICIDE CONTAMINATION IN CATTLE MILK AND FEEDSTUFF

Study to evaluate the difference in milk production in two groups of lactating cow's when one group was fed on fishmeal and other was on wheat bran diets. They observed that high milk production was obtained from wheat bran due to low cost to benefit ratio. In another study, Tahir et al. (2002) reported that milk production increased when dairy cattle was fed on maize bran rather than when fed on wheat bran and rice bran in Pakistan. Singh et al. (2000) reported that milk production increased in dairy cattle when fed on straw rather than when fed on deviled rice bran. The adverse health effect on cattle while feeding industrial by-products have not been reported yet but there are evidences in previous literature that

improper storage of such products in the farm can cause several infections among cattle's because of eating molded feed.

Cattle are exposed to pesticides in a variety of ways including

- By Inhalation
- Through Dermal Penetration.
- By Fodder

Pesticide residues in feed and fodders may transfer into herbivores through food chain of the animals Prassad and Chaabra (2001). Due to the lipophilic nature of these pesticides, milk and other fat-rich substances are the key items for their accumulation.

These toxicants get into the human body through the food chain and cause serious health problems Tsiplakou et al.(2010).

1. **Pesticide contamination in milk**

Moreover, Indian dairy industry is facing emerging challenges and also has a great opportunity in global trade with respect to the World Trade Organization (WTO) era. The Sanitary and Phytosanitary (SPS) and Trade Barrier Treaty (TBT) agreement in the WTO have vastly changed the current international food trade scenario.

Studies on pesticide residues in milk and dairy products are constantly focused on searching for organochlorine pesticides HECK et al. (2007), KUMAR et al. (2005), NAG and RAIKWAR, (2008) due to its high persistence in the environment and its toxicity. However, the prohibition of its usage in 1985, in Brazil, created the need of searching for its most common replacers, organ phosphorus (OPP) and carbamate (CB) pesticides.

Due to the risk presented to public health by milk contamination with OPP and CB residues, this study aimed to search for the presence of these residues in water and feedstuff supplied to dairy cattle and in milk produced by these animals.

Milk contamination is of extreme concern since milk and milk products have a very special position in the diet of infants, children and elderly for whom these are considered as a perfect natural food Goulart et al. (2008) This point is of particular interest since young children may be highly exposed to pesticides and they may be and higher risk of adverse health effects because of their physiological characteristics Eskenazi et al. *(*1999). The presence of pesticide residues have been reported in the bovine milk Pagliuca et al.(2006) and Sharma et al.(2007), Darko and Acquaah (2008) and Nag and Raikwar (2008).

2. Pesticides contamination in cattle feedstuff

Pesticides can appear in milk due to several possible causes:

- The use of insecticides directly on dairy cattle for ectoparasitic control.
- Pasture, forage (fodder), or animal feed manufactured from plant material that has been treated withInsecticides.
- The use of insecticides in stables or dairy factories. The amount of pesticides in milk depends on variousFactors, such as the stability of the pesticide, its metabolic fate in such as the stability of the pesticide, its metabolic fate in animals and its mode of application or intake.

High level of pesticide residues in feedstuff from post-harvest treatment or by drift during commercial aerial application inhaled air or contaminated water. Feedstuff manufactured from plant material that has been treated during growing season with insecticides i.e. contaminated through feed, grass/hay use of insecticides directly on the cattle against disease vectors, use of insectides in stables for the treatment flies in milk processing for hygienictreatment against insects.

MAIN OBJECTIVES OF THE PROPOSED RESEARCH WORK

- To assess pesticide residues in selected pastured land.
- Seasonal assessment of pesticide residues in cattle feed.
- Seasonal assessment of pesticide residues in cattle milk.

5. REVIEW OF LITERATURE

1. PESTICIDES CONTAMINATION IN CATTLE MILK

Kara and Ince (2016) determined Organophosphate (OP) pesticides *via* consumption of milk and milk products induce hazardous effects on human health. Considering their risk to human health, the determination of organophosphate pesticide levels in dairy products is very important. Until now, there has been limited data available on the occurrence of organophosphate pesticides in dairy products in Turkey. The aim of the present study was to investigate the presence of Malathion and malaoxon residues in commonly consumed buffalo and cow milk products in the city of Afyonkarahisar, Western Turkey. For this purpose, 75 buffalo and 75 cow milk samples were collected from the city. Malathion and malaoxon levels were determined by means of liquid chromatography/ tandem mass spectrometry. In the study, neither Malathion nor malaoxan was detected in buffalo and cow milk samples. In conclusion, survey studies were performed continuously for organophosphate pesticide contamination in consumed milk samples and products in Turkey.

Shaker and Elsharkawy (2015) analyzed the raw buffalo milk samples from the agro industrial zone in Upper Egypt were analyzed for the presence of organochlorine and organophosphorus pesticides using gas chromatography-mass spectroscopy. Five organochlorine pesticides namely, alachlor, dieldrin, hexchlorobenzene, lindane and methoxychlor and three organophosphorus pesticides chlorpyrifos, malathion, and parathion-methyl were detected in the milk samples. In 44% of the samples, the concentrations of lindane and malathion residues exceeded tolerance levels set by the European Commission (EC) in 2008. In addition, the concentrations of chlorpyrifos, methoxychlor, and hexachlorobenzene residues exceeded the 2008 EC maximum residual limits (MRLs) in 33%, 66%, and 88% of the examined samples, respectively. However, the levels of alachlor, dieldrin, and parathion-methyl residues were below EC MRLs. The results of this study confirm the risks of pesticide residues exposure that threaten consumer health in Egypt.

Chanda et al. (2014) reported pesticides are the compounds used to prevent organisms harmful to man. Depending upon the types of pest controlled, toxic property and chemical composition, pesticides are classified variously. Despite the fact that pesticides were used from very ancient time, the chronological consideration regards the discovery of DDT (1939) as the era of chemical pesticides and its development in the subsequent years. Initially, pesticides had tremendous use to improve quality of life and survival rate of man by providing sufficient amount of food and security against life threatening diseases. Beside this, the overuse of chemical pesticides imparts harmful effects on environment, ecosystem

and human health. These effects are intensified by the persistence of the pesticides in the environment and its mobility causing the toxicity to non-target species including man even in virgin area. Being aware of these damages, several measures have been taken that include the restricted use of most hazardous organochlorinated compounds along with the development and practice of eco-friendly approach of pest management for sustainable development.

Aslam et al. (2013) analyzed the ill effects of green revolution include residues of extensively used chemical pesticides in various environmental components. The present levels of organochlorine pesticide residues along with chemical composition in buffalo milk samples collected from different localities of Delhi. Milk monitoring can yield information about the kinds and quantities of pesticides in the environment as well as in our daily diet. In this study, the residue of three different organochlorine pesticides, namely Hexachlorocyclohexane (HCH), Dichlorodiphenyltri- chloroethane (DDT) and Endosulfan have been reported. Residues of Lindane exceeded the Maximum Residual Limit values in 50% of the samples is a cause of serious concern. The p,p'-DDT was detected in 70% of the samples with p,p'-DDE (dichlorodiphenyldichloroethylene) in 80% of the milk samples of different parts of Delhi state. DDD (Di- chlorodiphenyldichloroethane) another metabolite of p,p'-DDT was detected in 65% of the milk samples. The analysis indicates that DDT is the major contaminants in different parts of Delhi state. $\alpha$and β endosulfan were detected in 35% and 40% of the samples analyzed. The statistical correlation shows no significant correlation between chemical compo- sitions of the samples. The presence of multiple chemicals in virtually all samples of buffalo milk raises new questions about the possible toxicological impacts of chemical mixtures on an infant's developing nervous and immune systems and reproductive organs.

Iftikha et al.(2013) studied the transfer of organophosphate and pyrethroid pesticides in dairy cattle's milk when fed on agro-industrial by-product diet were assessed in this study. The transfer and accumulation of such pesticide in cattle fat tissue and milk was also assessed and the adverse effect on cattle's and a human health was also studied. For that purpose, about 80 milk and 30 diet samples were collected from various dairy farms. All samples were extracted with acids using "quick, easy, cheap, effective, rugged, and safe" (QuEChERS) method and analyzed through gas chromatography-mass spectrometry (GC-MS). The results show that about 40 and 20% of milk samples had greater content of cypermethrin+chloropyrifos and porfenofos than their maximum residue limits as suggested by World Health Organisation (WHO). Cypermethrin, chloropyrifos and profenofos were present at concentration greater than their maximum residue limits in mixed diet whereas

profenofos was completely absent in sugarcane khal and was present in traces in cotton khal but remained within the maximum residue limit. Nonetheless, transfer of residue of parent cypermethrin, chloropyrifos and profenofos to milk was not consistent with diet in all dairy milk samples. This revealed the contention that some other sources such as drinking or inhaling contaminated water or dust are also contributing to pesticide contamination in milk. Cancer potency factor for cypermethrine in children and adults remained within the recommended value. Generally, although pesticides residue in milk was not high enough to cause cancer risk in human, they might cause adverse health effect and delayed toxicity due to their long term accumulation and persistence within cattle's body.

Zhou et al. (2011) investigated the occurrence of persistent OCPs in 24 pooled breast milk samples collected from mothers from twelve provinces in mainland China. DDT was the most prevalent, followed by HCH and HCB, whereas levels of chlordane compounds, drins and mirex were lower. The mean contamination level of DDT, HCH and HCB in breast milk samples were 527.2, 231.8, 32.8 ng g-1 lipid, respectively. The mean concentrations of DDT, HCH and HCB in breast milk from rural and urban areas were: 539.3 and 629.2 ng g-1 lipid; 197.9 and 265.6 ng g-1 lipid; and 33.8 and 32.3 ng g-1 lipid, respectively. A positive correlation was observed between concentration of DDTs in human milk and consumption of animal origin food which suggested that this parameter could play an important part in influencing OCPs burdens in lactating women. The mean estimated daily intakes of different OCPs for breastfed infants were lower than the tolerable daily intake.

Abou Donia etal.(2010) studied on the accumulation of pesticide residues in milk and milk products in Egypt. Authors reported the mean residue levels of lindane, aldrin, heptachlor epoxide, chlordane and endrin as 46.0, 66.0, 64.0, 22.0 and 20.0 ng g-1 (fat basis), respectively in buffalo milk samples. The levels of DDT metabolites were 22.0, 6.0, 4.0, 60.0, 16.0 and 14.0 ng g-1 (fat basis) for p,p‟ DDT, o,p‟ DDT, p,p‟ DDE, o,p‟ DDE, p,p‟ DDD and o,p‟ DDD, respectively. The values for pesticide residues were exceeded the tolerance a level of FAO Lindane was reported highest in sample in comparsion of other pesticide.

Bulut et al.(2010) analyzed sixteen OCPs residues in kaymak and butter samples in showed that kaymak and butter samples were contaminated with 13 and 15 OCPs, respectively. The amount of Turkey. Their results), aldrin (12.34 ng g-1), dieldrin (12.69 ng g-1) and endosulfan sulphate (8.08 ng g-1) in butter were found to exceed the acceptable level set by the EU codex. Authors concluded that OCP residues in milk products could be a potential risk for public health due to their extensive consumption.

Melgar et al. (2010)monitored residue levels of seven OPs widely used as dairy cattle ectoparasiticides or in crops used for animal feed were determined in raw milk and infant formulas. A total of 312 samples were collected (70 from infant formulas and 242 from raw milk). The frequency of total samples containing detectable levels of OPs residues was 6.73% in total milk and 8.67% in raw milk. The highest percentage incidence measured was for dichlorvos (5.78%) followed by coumaphos (2.06%) and parathion methyl (0.83 %). The range of positive results was calculated to be between 5.0 and 220.0 ng g-1. No residue was detected in the infant formulas.

Abhilash and Singh (2009)studied agricultural development continues to remain the most important objective of Indian planning and policy. In the process of development of agriculture, pesticides have become an important tool as a plant protection agent for boosting food production. Further, pesticidesplay a significant role by keeping many dreadful diseases. However, exposure to pesticides both occupationally and environmentally causes a range of human health problems. It has been observed that the pesticides exposures are increasingly linked to immune suppression, hormone disruption, diminished intelligence, reproductive abnormalities and cancer. Currently, India is the largest producer of pesticides in Asia and ranks twelfth in the world for the use of pesticides.5% samples were contaminated with one or more of the investigated OCPs. DDT complex was the most frequent.

Hernandes et al.(2009) analyzed 69 samples of milk for the determination of deltamethrin and cypermethrin in were detected in 14 samples (21 %) at concentrations up to 12.0 ng g-1 and deltamethrin was detected in 3 samples at mean levels of 12.0 ng g-1. These results indicated that the practices used in the study region had as consequences the presence of pyrethroid residues in the milk produced. Therefore, in order to guarantee the quality of this important food the produced milk should be monitored and the producers should be oriented to use good agricultural practices aiming to improve the sanitary management of the milk producing cows.

Polder et al.(2009) determined PCBs and OCPs in 423 breast milk samples from women living in Norway. The samples were collected in six counties representing South, Central and North Norway in years 2002–2006. Their initial results showed significantly lower levels of organochlorines in breast milk from ethnic Norwegians (N=377) compared to ethnic non-Norwegians (N=46). Median concentrations (range) of PCBs, p,p‟ DDE, HCB, β-HCH and oxychlordane in breast milk of the Norwegian women were 103 (34– 450), 41 (5.4–492), 11 (3.6–24), 4.7 (0.9–37) and 2.8 (0.5–16) ng g-1 lipid weight, respectively.

Salem et al.(2009) foundthe use of aldrin, dieldrin, endrin, heptachlor and hexachlorobenzene (HCB) has been banned in Jordan officially in 1981 and of DDT in 1995. However, residues of such compounds can still be found in the environment and in foodstuffs. The presence of these OCPs residues in 233 dairy product samples; comprising milk, butter, cheese, labaneh and yoghurt collected from Jordan was determined. The results indicated that 9 % (21/233), 8.5% (20/233), 6.0% (14/233) and 2.1% (5/233) of the examined samples were contaminated with β-HCH, p,p‟ DDE, α-HCH and γ-HCH, respectively. Heptachlor and α-endosulfan were only present in less than 2% of the analyzed samples. None of the samples revealed the presence of aldrin, o,p‟ DDD, p,p‟ DDD, o,p‟ DDE, o,p‟ DDT, p,p‟ DDT, dieldrin, β-endosulfan, endrin and HCB at their detection limits. The order for the contamination in the analyzed dairy products was labaneh > cheese > yoghurt > butter > milk.

Gazzotti et al.(2008) measuredthe residues of 15 organophosphorus pesticides used as dairy cattle ectoparasiticides or as insecticides in crops used for animal feed. For this purpose a previously devised method was improved and validated. The samples were collected directly from tank trucks during delivery of3, 974 tonnes of raw milk at nine Italian dairy plants.Approximately 4.4% of the 298 samples analyzed contained residues only in traces. The main pollutant waschlorpyriphos.

Nag and Raikwar (2008)carried out monitoring India to evaluate the status of organochlorine pesticide (OCP) residues. Out of a total of 325 samples 20663.38%) were contaminated with residues of different OCPs. The average concentration oftotal HCH was 0.162 mg/kg. Among the different HCH isomers the frequency of occurrenceof α-isomer was maximum followed by δ-, γ- and β. Endosulfan (α, β, sulfate) was detectedin 89 samples with meanconcentration of 0.0492 mg/kg while total DDT comprising of DDT,DDE and DDD was present in 114 samples having mean concentration of 0.1724 mg/kg.

Zietz et al. (2008) collected 4314 human milk samples in the years 1999–2006 and analyzed for persistent organic pollutants. A clear downward trend of median total PCBs, DDT, β-HCH and HCB values in all participants and also in different selected subgroups were observed. The median values of total PCBs in the year 2006 was 182.5 ng g-1 lipid, that of DDT 81.5 ng g-1 lipid, β-HCH 11.6 ng g-1 lipid and of HCB 22.9 ng g-1 lipid. There were reductions between 40.9% and 47.1% compared to the year 1999. Among other influencing factors, median concentrations of total PCBs, DDT, β-HCH and HCB showed a clear rise with increasing age of mothers whereas an increasing number of breastfed infants per mother

led to a decrease. Authors concluded that the known declining trend of important xenobiotic substances in human milk of German mothers has continued.

Bai et al. (2006a)analyzed 240 samples of six kinds of dietary products, including cereals, vegetables, fruits, meat, fish and milk collected from local market of Shaanxi Province, China. It was reported that HCH residues were found in 96.7% of meat samples and 80.0% of milk samples with mean residue level of 6.2 and 3.8 ng g-1, respectively. While DDT residue levels were detected more in milk samples followed by meat with mean level of 11.8 and 9.2 ng g-1, respectively. In milk samples residues of α-HCH were detected in maximum number of samples.

Kumar et al. (2006) monitored milk sample for the present pesticide residues. The farmers use huge amounts of pesticides to increase the crop productivity. Exposure of humans to these hazardous chemicals occurs directly in the fields and indirectly due to consumption of contaminated diet, or by inhalation or by dermal contact. The organochlorine pesticides are reported to be lipophilic and their presence in human milk and blood has been documented in different parts of the world. Blood and milk samples were collected from lactating women who were divided into four groups on the basis of different living standards viz residence area, dietary habits, working conditions and addiction to tobacco. The level of total organochlorine pesticides in blood ranged from 3.319mg/L—6.253mg/L while in milk samples it ranged from 3.209Mdash;4.608 mg/L. The results are in concurrence with the reports from other countries.

Pagliuca et al.(2006) determined the contamination in raw milk by the main OPs pesticides used in Italy and to evaluate the opportunity to start specific procedures of risk management along the milk production chain. The samples collected in four Italian dairy plants directly from the tank trucks during the delivering, were representative of 920 tonnes of raw milk. The isolation of the organophospahtes (acephate, chlorpyrifos, chlorpyrifos-methyl, diazinon methamidophos, methidathion, phorate and pirimiphos-methyl) was performed by liquid-liquid partitioning followed by clean-up with solid phase extraction. Among the 135 samples analyzed, 37 were positive in traces and 10 showed an OPs contamination ranging from 5 to 18 ng g-1. The higher results were recorded in the samples collected during the autumn-winter period. The main pollutants detected were acephate and chlorpyrifos. 3.0±10.0 ng g-1, while the mean concentration of dieldrin was 127.3±20.0 ng g-1.

Kumari et al.(2005)analyzed butter (45) and ghee (55) samples collected from rural and urban areas of cotton growing belt of Haryana for residues of OCPs, SPs and OPs. The

estimation was carried out by using multi residue analytical technique employing GC-ECD and GC-nitrogen phosphorus detector (NPD) systems equipped with capillary columns. Butter samples were comparatively more contaminated (97%) than ghee (94%), showing more contamination with OCPs from urban samples. About 11% samples of butter showed endosulfan residues above MRL value and 2% samples had residues of SPs and OPs each above their respective MRL values.

Misra et al.(2005) detected SPs residues in samples of cow milk, poultry egg and meat collected from Tarai (foot hills) and hilly regions of Kimono. The residues were estimated with the help of high performance liquid chromatography (HPLC). The mean values of deltamethrin residue of the positive samples in milk, eggs and meat in the whole Kumaon region were 68.8, 44.8 and 35.6 ng g-1, respectively, while the values for fenvalerate residue were 149.8, 162.7 and 16.4 ng g-1, respectively. The results confirm the use of SPs in Kumaon regions of Uttaranchal. However, none of the sample showed residual concentration above the MRL as per the guidelines of the FAO/WHO.

Wahab et al. (2004) analyzed fourty samples of fresh dairy cow milk collected from some villages at Qena and Sohag Governorates of Egypt. The results of the study revealed that 82contaminant and were found in 30% of milk samples followed by γ-HCH (lindane) and heptachlor as each of them was found in 22.5% of milk samples. Endrin was found in 17.5% and then dieldrin and heptachlor epoxide as each was found in 15% of milk samples. The mean concentration of DDT complex and HCH was 100.3±190.0 and 170±16.0 ng g-1, respectively. The mean concentration of heptachlor was 57.5±013.0 ng g-1. Heptachlor epoxide was found in a mean concentration of 5pesticide.

Ciscato et al.(2002) analyzed residue of pesticides in 132 samples of cow milk collected from bulk transports (38 samples of raw milk) and market (94 samples of pasteurized milk). These samples were analyzed by the multiresidue analytical method for pesticide contamination. The results showed that 0.76% of samples were contaminated with HCH (α-isomer) and 10.60% with endosulfan (α- and β- isomers). As per authors, endosulfan and HCH found in milk samples.

Battu et al.(2004) examined ninety two samples of liquid milk from Ludhiana, India, during 1999-2001 and revealed the presence of DDT in 6 (7.4%) samples and of these 2 samples were found to exceed the MRL of DDT fixed at 50 ng g-1 (on a whole milk basis). HCH residues were detected in 49 (53.3%) samples and constituted only γ-HCH. The MRL of lindane is fixed at 10 ng g-1 (whole milk basis) and all 49 liquid milk samples exceeded this value. These results were indicative of contamination of bovine milk with pesticide residues

as a result of the ban on the use of DDT and HCH in agriculture and public health programs. Similarly, analysis of 40 samples of butter showed the presence of DDT and HCH in 28 and 8 samples, respectively. However, none of the samples exceeded the MRL value of either DDT or any isomer of HCH. DDT residues comprised mainly p,p‟ DDE and p,p‟ DDD, whereas HCH residues were present as lindane in 6 samples and 2 samples revealed.

Salaset al.(2003)monitored Mexican milk samples for 13 OPs, which were widely used as dairy cattle ectoparasiticides or in crops used for animal feed. Four different milk brands with high distribution were collected biweekly during a 12 month period (n=96) in supermarkets. OPs pesticide residues were measured by gas chromatography with a flame photometric detector. It was reported that approximately 39.6% of the samples contained detectable levels of OPs pesticide residues. Eight samples contained residues exceeding established MRL and the OPs present in these samples were dichlorvos (five samples), phorate, chlorpyrifos, and chlorfenvinphos (one sample, respectively). Average residues of 13 OPs pesticides measured were below established MRLs ranging between 5.1 and 20.3 ng g-1.

Pandit et al.(2002) monitored 520 milk and dairy product samples of various brands from different cities in Maharashtra, India for OCPs. The measurements were made using a GC-ECD system. α-, β- and γ- isomers of HCH and p,p‟ DDT, p,p‟ DDD, p,p‟ DDE were detected in all the milk samples analyzed. Total HCH levels in milk and milk product samples were lower than total DDT levels use in agriculture practices, although legislation in Brazil does not permit the use of HCH since 1985 and endosulfan can be used only in a few crops. Organophosphorus, carbamates, pyrethroids, herbicides, and fungicides were not detected in cow's milk samples.

John et al.(2001) reported that the widespread application of pesticides in agriculture and public health programme can result in the accumulation of pesticides in the environment. Therefore, a survey was conducted during 1993-1996 to investigate the magnitude of contamination of bovine milk with OCPs residues from Jaipur City, Rajasthan, India. Milk samples i.e., dairy (toned and whole) and buffalo milk were collected seasonally and pesticide residues were assessed using GC with an electron capture detector (ECD). The results indicate that all the milk samples were contaminated with DDT and its metabolites DDE and DDD, α-, β-, and γ- isomers of HCH, heptachlor and its epoxide and aldrin. Seasonal variations of these pesticide residue levels were also observed in all the milk samples. Samples collected during winter season were found to contain higher residue levels as compared to other seasons.

Battu et al.(1989) studied Bovine milk samples collected from Ludhiana and Sangrur districts of Punjab, India, where DDT and HCH, respectively, had been sprayed for the control of malaria were analyzed for insecticide residues between September 1985 and June 1986. The average concentran of DDT residues in samples from the DDT sprayed area was 4-12 times higher than those in the corresponding samples from HCH sprayed areas. Mean levels of HCH residues in samples from the HCH sprayed area were greater than those in samples from DDT sprayed areas by factors of 2-11. Milk samples collected before and after applications of DDT or HCH for mosquito control also showed that indoor use of these insecticides results in a substantial increase in residue levels. About 74% of the samples analyzed contained DDT residues above the extraneous residue limit of 50.0 ng g-1.

Witt et al.(1966) found two common metabolites DDE and DDD (dichlorodiphenyl dichloroethane) of DDT in cow's milk when they fed cows with DDT in feed and considered that animal feed is the major source of exposure to the milk animals.

2. REVIEW CATTLE FOODSTUFF

Mahmoud and Hayajneh (2015) studiedthe pesticides which are chemicals used to increase the productivity of plants which are used later on as feed for animals, have been intensively used by farmers, the misuse of these chemicals can result in their presence in foodstuff of humans, people must be aware of this problem which can lead to serious health problems to humans, the current study measures the awareness of people in Taif region, Kingdom of Saudi Arabia towards this problem. 27% of the samples under study do not know that pesticides are harmful when consumed through contaminated vegetables and fruits. 23% of the samples do not know that such chemicals could be transferred to our kitchens through contaminated vegetables and fruits.

Ahmed et al. (2012) collected 200 dairy cows mostly the crosses between local and Holstein Friesian (LxF), Local and Sahiwal (LxSL) and also between Local, Holstein Friesian and Sahiwal (LxFxSL) to improve milk production performance through nutritional interventions. Rice straw based feeding intervention was composed mainly of Urea Molasses Straw (UMS) without green forage in intervention was composed mainly of Urea Molasses Straw (UMS) without green forage in monsoon, reduced quantities of legume forage in winter and increased amount of non-legume forage in summer. Feeding UMS instead of rice straw increased DCP and ME intake and slightly increased feed cost in monsoon. In winter, legume

forage supply was curtailed over that supplied traditionally and rice straw was increased which reduced DCP and ME intake and also feed cost slightly. The tervention in summer season was to reduce rice straw and concentrates and to increase the supply of green grass. Results showed that DM, DCP and ME intake were improved in summer and feed cost reduced. Present study was found effective for the improvement of nutritional status and productivity of dairy cows in Bangladesh.

Javed et al. (2012) reported Wheat bran, a by-product of wheat milling industry, is the outermost covering of wheat grain. It has relatively diverse application in food, feed, medicine and fermentation industries due to its richness in carbohydrates (mostly fibers), protein and fats which makes it an important dietary element. Its importance from medical view point is well documented especially in reducing blood plasma cholesterol and prevention of cardiovascular diseases and certain cancers, that is, colon cancer. In fermentation industry, wheat bran can be used as a substrate for production of a range of products such as biomass, enzyme, biofuel and production of other metabolites. In short wheat bran has immense applications and market value which makes it so important to be considered as brown gold.

Fagnani et al. (2011) carried out considering acute and chronic toxicity effects on human and animal health caused by pesticide residues in food, to analyze organophosphate (OP) and carbamate (CB) in feedstuff and water destined for dairy cattle, as well as in the milk produced by these animals, through gas chromatography (GC). In the Agreste region of Pernambuco, Brazil, 30 raw milk samples and all components of the animals' diet were collected from several farms. Out of the 30 milk of milk analyzed, six (20%) were contaminated with OP, five (16.7%) with CB, and one sample with both pesticides. From 48 analyzed feed samples, 15 (31.25%) were contaminated with residues of OP, six (12.50%) with CB, and one sample was contaminated with both pesticides. Out of 16 water samples analyzed, six (37.50%) were contaminated with OP residues, but non with CB. In four dairy farms the pesticides detected in milk were compatible with the active principles found in water and/or foodstuff, suggesting them to be the source of contamination.

Nag and Raikwar (2011) determined the frequency of occurrence and contamination levels of OCPs residues in different kinds of animal concentrate feed and straw samples collected from Bundelkhand region of India. Out of 533 total samples, 301 (56.47%) samples were found to be positive containing residues of different OCPs like HCH isomers, DDT complex, endosulfan and dicofol. About 60%samples were found contaminated with DDT and HCH in

case of mustard seed cake, cotton seed cake, linseed cake, gram chunni, pea chunni and rice chunni. Among different HCH isomers, the mean concentration of β-HCH was highest and total HCH varied from 10.0 to 306.0 ng g-1. The concentration of total DDT ranged between 16.0 and 118.0 ng g-1 and the pp isomers were more frequently encountered than their op counterparts. Endosulfan was also found in some samples in concentration ranging from 9.0 to 237.0 ng g-1, but dicofol was recorded in few samples.

Tsang et al.(2011) investigated the body burdens of persistent organic pollutants in residents of Hong Kong. Twenty nine human milk samples and 21 human blood and cord blood samples collected from 2005 were analyzed for polyaromatic hydrocarbons (PAHs), OCPs and PCBs levels. Higher levels of PAHs, DDTs and PCBs were detected in human milk samples when compared to maternal serum and cord serum (PAHs: milk: 1981 ng g−1 lipid, maternal serum: 1461, cord serum: 1158; DDTs: 3099, 1934, 1556; PCBs: 49, 41, 40). p,p‟ DDE and p,p‟ DDT were the only metabolites of DDT detected in three types of human tissues. The significant correlations were observed between total DDTs and p,p‟ DDE in human milk with consumption of freshwater and marine fish and maternal age. The estimated daily intakes of DDTs by infants indicated that 7 out of 29 of the human milk samples exceeded 20 ng g-1day−1; the tolerable daily intake (TDI) proposed by the Health Canada guideline in terms of DDTs levels.

Fernandez-Alvarez etal. (2010)analyzed the target plant protection products in 27 cattle feed samples collected in 23 dairy farms.In two feed samples lindane levels (5.7 and 5.6 ng g-1) were exceeded the MRL, while one sample of feed exceeded the MRL for fenitrothion and chlorpyrifos with levels of 14 ng g-1 and 71 ng g-1, respectively. Chlorpyrifos was also detected in another four samples at lower concentrations (0.2–8.0 ng g-1) and fenitrothion in another six (2.6–9.3 ng g-1). Other target plant protection products detected were endrin aldehyde (9.4–12.1 ng g-1), alachlor (3.4 ng g-1), α-endosulfan (1.1–5.3 ng g-1), β-endosulfan (1.9–3.8 ng g-1), endosulfan sulphate (1.7–3.8 ng g-1) and tetramethrin (1.8–17 ng g.

Rai et al.(2008) determined the residues of carbaryl pesticide in surface water, milk, poultry eggs, and meat, fodder and grain samples collected from differentcentres of Kumaon region of State of Uttarakhand. The highest mean residual concentration (mg/g) of carbaryl was 0.0527 in water followed by 0.0541 in meat, 0.0506 in poultry eggs, 0.0453 in milk, 0.0435 in food grain, and 0.0344 in green fodder. A total of 19.51% of fodder samples from Kumaon region were positive for carbaryl residues with mean value 0.0344 mg/g. A maximum

concentration of 0.0535 mg/g and a minimum of 0.0219 mg/g were detected in samples of Kichha and Kashipur, respectively. The highest percentage of 50% positive samples was reported from Pantnagar and Kichha's center, whereas, samples from Haldwani, Ranikhet and Nainital were found negative for the carbaryl residues. The pesticide residues in fodder might have accumulated as a result of direct application of pesticide, uptake from soil and/or contamination due to drift during spraying.

Kan et al. (2007) determined toxic substances such as dioxins, mycotoxins, heavy metals, pesticides, veterinary drugs and polycyclic aromatic hydrocarbons are almost ubiquitous in the environment. Thus, they are also present in ingredients for animal feed. Adequate risk management depends on knowledge of absorption, metabolism, carry-over and toxicological profile of these substances and on practical measures to reduce especially the latter two. Generally, toxic substances are metabolized before or after absorption through the intestinal tract. Depending on their physico-chemical characteristics, some substances are metabolized into naturally occurring and generally harmless constituents. Most veterinary drugs and feed additives fall into this group. Other substances are persistent and remain in the animal and in animal products, like dioxins. Heavy metals are not metabolized at all. Some metals irreversibly are bound to body tissues, *e.g.* lead to bone or cadmium to kidneys.

Sharma et al.(2005) collected fifty animal feed concentrates samples from Karnal (Haryana), India and analyzed them for 60 different organochlorine, organophosphate, carbamates and synthetic pyrethroids pesticide residues. They extracted the samples by liquid-liquid partitioning and cleaned by gel permeation chromatography. The estimation of residues was done by using GC with ECD and NPD detectors. The results indicated the presence of phorate, monocrotofos, dimethoate, diazinon, carbaryl, chlorpyrifos and p,p‟ DDT with the mean levels of 7.0, 8.0, 15.0, 11.0, 2.0, 9.0 and 7.0 ng g-1 (fresh weight basis), respectively. Endosulfan and dicofol residues were detected in one and two samples, respectively.

Sughathy et al.(2002) monitored chlorinated hydrocarbon insecticide and chlorpyrifos residues in animal feed materials (such as cottonseed, fodder maize, fodder sorghum, teli grass, maize grain and cumbu grain) sampled from Coimbatore, Tamil Nadu, and India at weekly intervals from November 2001 to March 2002. Authors reported that some of the animal feed samples were contaminated with endosulfan sulphate and chlorpyrifos residues. Residues of α-HCH were detected in cottonseed, fodder maize, fodder sorghum and cumbu grain, while fodder maize, fodder sorghum, teli grass and cumbu grain were contaminated with γ-HCH. B-HCH was detected only in fodder maize from below detectable level (BDL)

to 132.0 ng g-1. All the samples were free from α- and β- endosulfan residues. However, endosulfan sulphate was detected in all the animal feed samples. None of the samples were contaminated with any of the organochlorine and chlorpyrifos residues above the MRL.

Tahir et al. (2002) studied effect of feeding concentrate based on different barns on the performance of Holstein Friesian cows. Twelve Holstein Friesian cows of second and third lactation were randomly distributed into three equal groups. The animals of group A were fed on a concentrate mixture containing 30% maize bran, group B animals on a concentrate mixture containing 30% de-oiled rice bran; whereas, the animals of group C on a concentrate mixture containing 30% wheat bran. Maize fodder was given ad-lib to the cows of all groups. The feeding trial lasted for 60 days. The intakes of dry matter (DM), crude protein and TDN were same in all groups. The milk fat and total solids were not affected by experimental rations. Significant variation was determined in solids-not-fat of milk produced by animals fed on different experimental rations. The average daily milk yields were 18.05, 12.87 and 14.65 L in groups A, B and C, respectively. The animals of group A showed statistically better milk production which indicates the maize bran feed was nutritionally better and can be successfully utilized in the dairy cattle ration to sustain production.

Prasad and Chhabra (2001) reported that cereal grains, oil-cakes, brans, legume and non-legume forages and crop used for animals feed were contaminated with OCPs residues. The order of contamination in concentrates and roughages was HCH > endosulfan > heptachlor > DDT > aldrin. The HCH content was highest in concentrate mixture followed by maize, groundnut cake, wheat bran, rice bran, cotton seed-cake and barley in concentrates and in roughages like maize, oat, sorghum, berseem fodders, wheat straw, lucerne and paddy straw in the same order. It was concluded that all the tested animal feed resources were contaminated with one or theother component of OCPs residues and there was likelihood that these may be contaminating animal products.

Nath et al.(2000)detectedHCHandDDT in almost all the samples of fodder collected from the dairy farms in Bangalore, Karnataka, India. Endosulfan was detected in some samples. The source of high levels of DDT detected in the crops from a few farms up to 4310.0 ng g-1 on dry basis was found out to be the sewage water used for irrigation. DDT was not present in the drinking water supplies of these farms but HCH was detected. The organochlorine pesticides were present mostly in trace levels in the soil samples tested.

Mukherjee and Gopal (1996) analyzed different constituents of animal feed concentrate for pesticide residues which were extracted by exhaustive blending and further clean up by

sulphuric acid treatment. Total HCH residue levels in wheat straw, paddy straw, rice husk, gram straw, mustard cake, cotton seed cake, wheat grain, gram grain and maize grain were 112.0, 35.0, 904.0, 1860.0, 7.0, 91.0, 110.0, 248.0 and 10.0 ng g-1, while the levels of DDT were 63.0, ND, 251.0, ND, 69.0, 72.0, 35.0, ND and ND ng g-1 , respectively. The levels of HCH and DDT in green fodder were reported as 16.0 and 26.0 ng g-1, respectively.

Dikshith et al.(1989)analyzed thirty two samples of animal feed collected from local market and their findings indicate that γ-HCH was found in all the samples. The mean concentration of total HCH was 393.0 ng g-1 which was 3 fold higher than the γ -HCH. Residues of p,p‟ DDE, o,p‟ DDT, p,p‟ DDD and p,p‟ DDT were also present in all the samples, total DDT content was reported to be 236.0 ng g-1. The mean concentration of p,p‟ DDE and p,p‟ DDD were higher than those of other DDT metabolites like p,p‟ DDT and o,p‟ DDT. The level of aldrin was found 156.0ng g-1. Endrin one of the most toxic OCPs was also detected at level of 20.0 ng g-1 in all the feed samples while endosulfan was detected at level of 29.0 ng g-1.

6. RESEARCH GAPS IDENTIFIED IN THE PROPOSED FIELD OF INVESTIGATION

1. There is a lot of work carried out on pesticides analysis in different environmental area like soil, water, animals etc.

2.Number of Institution and organization like IndianAgricultural ResearchInstitute ,New Delhi, Pesticides Research Institute , National Institute of Environmental Health Science, Ministry of Agricultural Farmer Welfare, New Delhi , WHO, Geneva etc. are working on pesticide's.

3. Most of the work done so far is on pesticide inmilk, animal feedstuff but there is less work on seasonal crop in India and particularly Ajmer is untouched behalf of the pesticide research monitoring.

4. The Ajmer area is important for the milk- production but excessive use of pesticide is contaminating agriculture land and ground water .These have not been studied from animal feedstuff and domestic animal milk point of view. The pesticide residues in milk & feedstuff have also not been carried out for such arid and semi-arid condition.

7. MAJOR HYPOTHESIS

1. Possibilities of dietary transfer of pesticide to cattle milk from feedstuff are high.
2. Accumulation of pesticide in cattle milk is likely to occur.

8. RESEARCH METHODOLOGY

Ajmer has suitable climatic conditions for production of range of milk. Pesticides are being used by farmer's at large scale to avert the crop damage by insects and pests. This study has been conducted to determine the contamination level of pesticides in domestic cattle's milk and their feedstuff. The present research study is will based on library, field and laboratory research. Samples are collected from the study area. The sample of milk and feedstuff is collected from the study area. The sample will be collected from the different-different villages near about agriculture land in the study area and will be analyzed for different – different pesticide in milk of cattle's and their feedstuff.

- For the present study, GCused pesticide Analysis.
- Pesticides analyzed by using Gas Chromatography (model no) in the department of Environment science, Gas Chromatograph.
- Gas chromatographic analysis begins with injection of the samples in to the GC with syringe.
- The sample is the first vaporized at high temp (200-350) in the injection part and then forced on the separating column by carrier gas, (viz, nitrogen, heliumetc.).
- Gas chromatography mass spectrometry (GC/MS) instrumentation is a powerful tool for the separation and identification of volatile and semi-volatile compounds, including many OP pesticides. Gas chromatography coupled to a triple quadrupled mass spectrometer (GC-MS/MS) allows for even greater separation, as the mass spectrometer is operated in the Multiple Reaction Monitoring (MRM) mode with two stages of mass analysis.

PHASE 1:

1.Collection of literature on the methodology.

2.Standardization of methods.

3.Selection of sites for sampling.

4.Experimental set up for sample test.

PHASE 2:

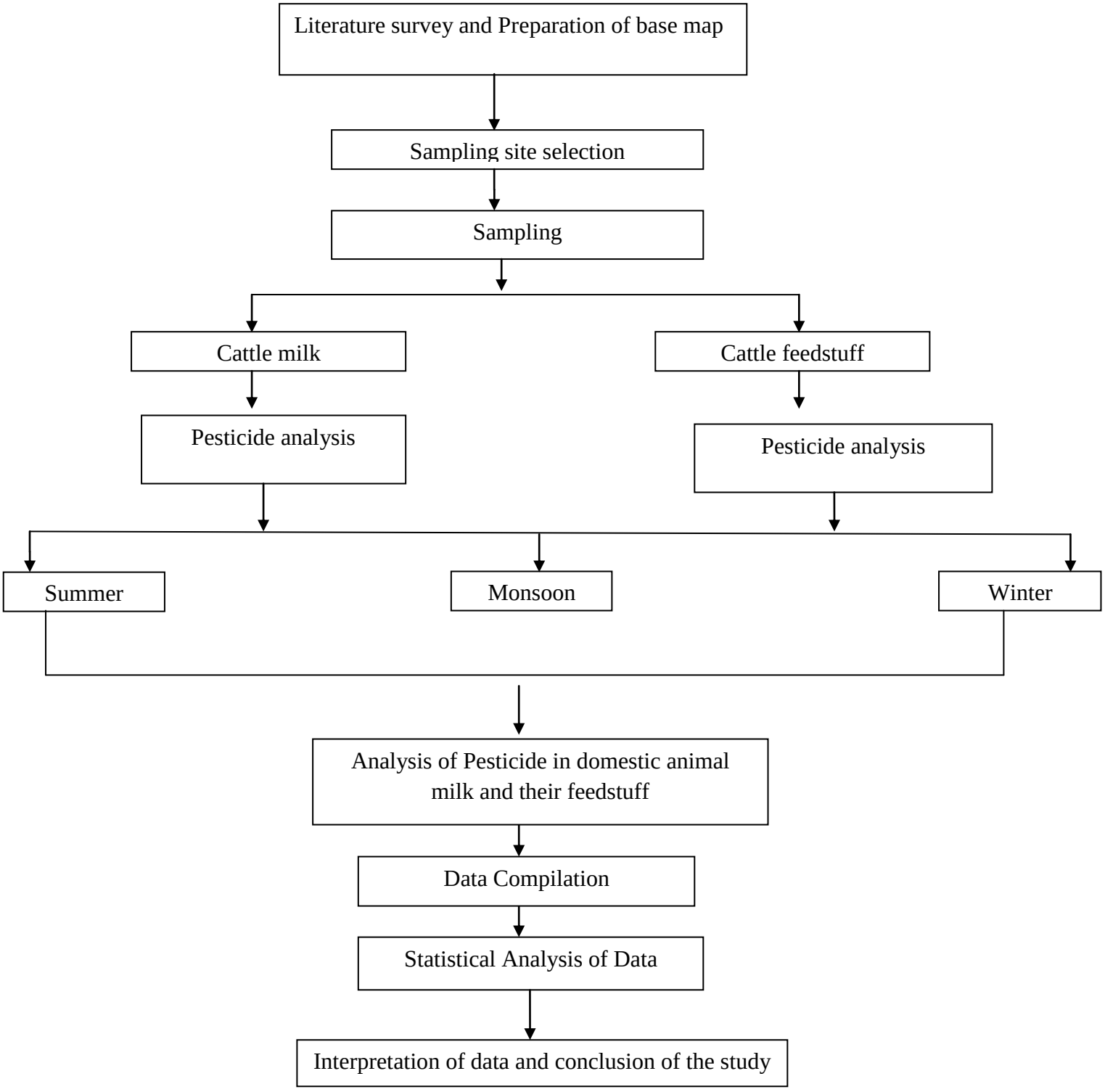
1.Seasonally analysis for detection of pesticide in cattle milk sample.

2.Seasonallyanalysis of feedstuff sample for pesticide concentration.

PHASE 3:

1. Tabulation of Results and Discussion.
2. Writing and typing of the thesis.

WORK PLAN OF THE RESEARCH WORK



FACILITIES AVAILABLE FOR THE RESEARCH WORK

All the necessary facilities required for my proposed study are available in the Department. The routine instruments for analysis of Pesticide determination and various parameters are readily available. The department has such basic infrastructures/instruments listed below-

1. Gas Chromatographic (GC)
2. UV Spectrophotometer
3. Sensitive Electronic Balances
4. Ovens, Incubators, Heaters, Distillation Units
5. Refrigerators
6. Centrifuges
7. Hot plates
8. Stirrers
9. Computers, Laser printers, Scanners etc

Beside above sophisticated instruments, the department has varied range of glassware's and chemicals required for analysis.

Signature

Head

Department of Environmental Science

M.D.S. University, Ajmer

विभागाध्यक्ष
पर्यावरण विज्ञान विभाग
म. द. स. विश्वविद्यालय
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Signature

Head
Environmental Science
University, Ajmer

Department of
M.D.S.

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Pratima Yadav 15/09/2016

Signature of the Candidate with date

Outline Approved

15/9/16

Name and Signature of supervisor with date & seal

Prof. Subroto Dutta
Department of Environmental Science,
M.D.S. University

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